UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL
ST/SOA/SER.J/16
27 November 1974
Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM [1]

No. 16

DETERMINATION OF THEBAINE IN PAPAVER BRACTEATUM [2]

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1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

GE. 74-12056

In the course of our work on the extraction of poppy capsules, we have developed analytical methods for the quantitative determination of opium alkaloids, including thebaine.

These methods are based on preliminary separation of the alkaloids by thin-layer chromatography, after which the alkaloids are traced under shortwave UV-light. After removal from the TLC-plate, the alkaloids are eluted from the absorbent. An aliquot of the eluate is treated with a reagent and the absorbance of the resulting solution is measured.

In the case of thebaine, the separation is carried out on silica gel. After elution with methanol, the method of Sakurai[3/] is used for thebaine determination.

Equipment

The sample solutions are applied on the TLC-plate by means of a 50 microlitre Hamilton pipette. For the detection of the alkaloid bands, a Camag shortwave UV-lamp (254 nm) is used.

The alkaloid bands are scraped off by means of a bent spatula. The silica gel is transferred into reagent tubes with a vacuum zone collector which consists of a G-3 glass filter (Ø 1 cm) provided on the upper side with a stopcock. The stopcock is connected with a vacuum pump and the silica gel is collected on the under side of the filter plate. The silica gel is transferred to reagent tubes by closing the stopcock and gently tapping the filter against the inner wall of the tube. A Zeiss Spectrophotometer type PM Q II is used for the absorption measurements.

Chemicals and reagents

All chemicals are of reagent grade. The solutions of 4-aminoantipyrine and potassium ferricyanide are prepared daily. The thebaine content of the standard thebaine HCl solution is determined by non-aqueous titration with perchloric acid. The standard solution of thebaine HCl, containing 0.6% (w/v) thebaine calculated as the base, is stored at 5°C.

3/ H. Sakurai, J. Pharm. Soc. Japan, 80, 909 (1960)

Extraction methods

Ground poppy capsules are mixed with 1.6 times v/w ammonia 0.35 N. After standing for 30 minutes, the mixture is extracted with chloroform. The residue from the chloroform extract is taken up in dilute acetic acid. For the analysis of Papaver bracteatum, the acetic acid solution is used as the starting solution for the chromatographic separation. In the case of a low thebaine content and extraction of a relatively large quantity of plant material, the acid solution is extracted at pH 11-13 with toluene. The toluene extract is shaken with dilute acetic acid and this aqueous extract is used for the thebaine analysis.

An alternative method is a soxhlet extraction of the capsule-ammonia mixture with toluene for 10 hours. In this case, the toluene extract is evaporated and the residue treated with ethanol. The ethanol solution is filtered through a glass filter and the filtrate is used for the thebaine analysis.

Description of the analytical method

Extraction of Papaver bracteatum capsules

One gram of ground capsules is treated with 1.6 ml ammonia 0.35 N. After leaving for 30 minutes, the mixture is shaken for 1 hour with 10 ml chloroform. The material is filtered and the extraction is repeated twice with fresh portions of 10 ml chloroform. The extracted papaver material is discarded.

The combined chloroform extracts are evaporated under reduced pressure at a moderate temperature. The still warm residue is dissolved in 3 ml aqueous acetic acid 0.5 N. The solution is quantitatively transferred to a 5 ml volumetric flask by the addition of two portions of about 1 ml acetic acid 0.5 N. The volumetric flask is filled to the mark and the extract is homogenized.

Chromatography

On a silica gel plate GF_{254} (20 x 20 cm) lines are drawn with a pencil making 5 rectangles of 11.5 x 3.8 cm. At the sides however, strips with a breadth of 0.5 cm are drawn which will not be used.

The solutions are applied 1.5 cm from the lower edge of the plate forming a band 3.4 cm wide. Formation of the band can be effected by the application of small drops half overlapping each other. Widening of the band can be prevented by drying with a gentle stream of nitrogen during application. In this way, a band is obtained not broader than 7 mm. If the rectangles are numbered 1 to 5, from left to right, the solutions are applied as follows:

1 : no solution (blank)

2 and 3: 40 microlitres sample solution

4 and 5: 40 microlitres of thebaine standard 0.6%

Note: For the analysis of only one sample, two plates are used to ensure having a sufficient number of blanks and standards.

The chromatograms are developed with a mixture of cyclohexane-diethylamine (9 + 1) and dried at room temperature for 30 minutes. The thebaine bands are marked under shortwave UV and scraped off and collected with the vacuum zone collector. In rectangle 1 a band is drawn with about the same width as the thebaine bands and this is scraped off.

The silica gel is transferred to round bottomed centrifuge tubes and 3 ml methanol is added. The tubes are closed with plastic stoppers and, after careful mixing, they are placed in the dark until the next day.

Colorimetry

The tubes are shaken again and then centrifuged. From the supernatant fluid, 2 ml is carefully pipetted into round bottomed 15 ml centrifuge tubes.

The tubes are placed in a boiling waterbath until the methanol has completely evaporated. 2 ml HCl 3.5% (w/v) is added to the residue in the tubes. After the residues have dissolved, the tubes are closed with glass beads, placed in a boiling waterbath for exactly 25 minutes and then cooled under a stream of tap water. 2 ml 4-amino antipyrine solution 0.375% (w/v)

and 1 ml potassium ferricyanide solution 0.6% (w/v) are added and, after exactly 1 minute, 2 ml ammonia 10% (w/v). The tubes are closed again with the glass beads and heated in a boiling water bath for exactly 5 minutes. After cooling, 1 ml ethanol is added. The tubes are closed with plastic stoppers and the contents are mixed well. The extinction of the resulting solutions is measured at 435 nm against water.

Calculation

The thebaine content of the original sample is calculated as follows:

$$\frac{0{,}6\ V\ (E_s - E_b)}{W\ (E_{st} - E_b)} = \quad \% \text{ thebaine}$$

Where: E_s = extinction measured for the sample
E_{st} = extinction measured for the standard
E_b = extinction of the blank
V = the total volume of the sample solution in ml
W = sample weight expressed in grams.